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☐ 1: Biosci Biotechnol Biochem 1995 Mar;59(3):382-7[Related Articles, Links](#)**High level secretion by *Saccharomyces cerevisiae* of human apolipoprotein E as a fusion to *Rhizomucor* rennin.****Nomura N, Yamada H, Matsubara N, Horinouchi S, Beppu T.**

Department of Biotechnology, University of Tokyo, Japan.

As the first step for production of human apolipoprotein E (hApoE) in *Saccharomyces cerevisiae*, the hApoE cDNA was cloned in *Escherichia coli*, on the basis of the nucleotide sequence reported previously. When the hApoE cDNA including its pre-sequence-encoding region was expressed under the control of the GAL7 promoter, no protein immunoreactive with anti-hApoE antibody was detected either in the culture medium or inside the cells. For efficient production and secretion of hApoE in *S. cerevisiae*, the mature hApoE-encoding region was fused to the prepro-sequence region of *Rhizomucor* rennin (MPR) and to the whole MPR gene including its prepro- and mature-MPR regions. When the fusion gene consisting of the prepro-sequence-encoding region and hApoE regions was expressed in *S. cerevisiae*, no protein reactive with the anti-hApoE antibody was detected in any fraction of the yeast cells, probably due to rapid degradation of the hApoE protein by yeast proteases. On the other hand, when hApoE was expressed as a fusion to the whole MPR protein, a considerable amount of the fused protein was secreted into the medium. The prepro-sequence of MPR was correctly processed from the fused protein in the medium by autocatalytic activity of MPR and by a protease(s) of the host cell. (ABSTRACT TRUNCATED AT 250 WORDS)

PMID: 7766173 [PubMed - indexed for MEDLINE]

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Mar 6 2003 10:50:17

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Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc, if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: \_\_\_\_\_

Inventors (please provide full names): \_\_\_\_\_

Earliest Priority Filing Date: \_\_\_\_\_

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Online Time: <u>6</u>	Other _____	Other (specify) _____

09/402,488 SEARCH RESULTS/HISTORY

(FILE 'HOME' ENTERED AT 10:21:52 ON 12 MAR 2003)

FILE 'MEDLINE, AGRICOLA, CAPLUS, BIOSIS, EMBASE, WPIDS' ENTERED AT  
10:22:43 ON 12 MAR 2003

L1 6112 S (CHYMOSIN OR RENNIN OR PROCHYMOSIN OR PREPROCHYMOSIN OR (EC (

L2 2294568 S FUSION OR FUSED OR LINK?

L3 408 S L1 AND L2

L4 11710 S (PROPEPTIDE OR PRO-PEPTIDE)

L5 9 S L4 AND L3

L6 5 DUP REM L5 (4 DUPLICATES REMOVED)

FILE 'STNGUIDE' ENTERED AT 10:37:01 ON 12 MAR 2003

=>

09/402,488 SEARCH RESULTS/HISTORY

YOU HAVE REQUESTED DATA FROM 5 ANSWERS - CONTINUE? Y/(N):y

L6 ANSWER 1 OF 5 WPIDS (C) 2003 THOMSON DERWENT  
 ACCESSION NUMBER: 2001-226621 [23] WPIDS  
 DOC. NO. NON-CPI: N2001-161041  
 DOC. NO. CPI: C2001-067636  
 TITLE: Producing **chymosin** in seeds of plants such as  
 rice, flax, rape seed, by transforming plant cell with a  
 nucleic acid encoding **chymosin** operably  
 linked to transcription regulator and terminator  
 sequences.  
 DERWENT CLASS: C06 D13 D16 P13  
 INVENTOR(S): BOOTHE, J; KEON, R G; SHEN, Y; VAN ROOIJEN, G  
 PATENT ASSIGNEE(S): (SEMB-N) SEMBIOSYS GENETICS INC  
 COUNTRY COUNT: 95  
 PATENT INFORMATION:

PATENT NO	KIND	DATE	WEEK	LA	PG
WO 2001014571	A1	20010301	(200123)*	EN	56
RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ NL OA PT SD SE SL SZ TZ UG ZW					
W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CR CU CZ DE DK DM DZ EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW					
AU 2000066780	A	20010319	(200136)		
EP 1216306	A1	20020626	(200249)	EN	
R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT RO SE SI					

APPLICATION DETAILS:

PATENT NO	KIND	APPLICATION	DATE
WO 2001014571	A1	WO 2000-CA975	20000823
AU 2000066780	A	AU 2000-66780	20000823
EP 1216306	A1	EP 2000-954228	20000823
		WO 2000-CA975	20000823

FILING DETAILS:

PATENT NO	KIND	PATENT NO
AU 2000066780	A Based on	WO 200114571
EP 1216306	A1 Based on	WO 200114571

PRIORITY APPLN. INFO: US 1999-378696 19990823

AN 2001-226621 [23] WPIDS

AB WO 200114571 A UPAB: 20010425

NOVELTY - Production (I) of **chymosin** in a plant seed comprises,  
 introducing a chimeric nucleic acid molecule comprising a nucleic acid  
 sequence encoding a **chymosin** polypeptide operatively  
 linked to transcription regulator and terminator sequences into a  
 plant cell, growing the plant cell into a mature plant capable of setting  
 seed and obtaining seed containing **chymosin** from the mature  
 plant.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the  
 following:

(1) a plant seed comprising 0.5% (w/w) heterologously expressed  
**chymosin**; and

(2) a plant capable of setting seed comprising 0.5% (w/w) of  
 heterologously expressed **chymosin**.

USE - The method is useful for producing plant seeds, in particular  
 seeds of soybean, rape seed, sunflower, cotton, corn, tobacco, alfalfa,  
 wheat, barley, oats, sorghum, Arabidopsis thaliana, potato, flax/linseed,  
 safflower, oil palm, groundnut, Brazil nut, coconut, castor, coriander,  
 squash, jojoba and rice containing 0.5%, 1%, 2% or 4% (w/w)  
**chymosin** in the total seed protein (claimed).

ADVANTAGE - Plant seeds where **chymosin** accumulation levels  
 exceeds 0.5% (w/w) of total seed protein can be obtained by this method.  
 These high expression levels in plant seeds allow significant commercial  
 savings since the acreage of plants that need to be grown can be limited  
 and the amount of biomass that must to be subjected to extraction is  
 reduced. The amount of biomass processed is further limited due to the  
 relatively of low water content present in the plant seed. Further the  
 expression in plant seed offers flexibility in storage and shipment of

## 09/402,488 SEARCH RESULTS/HISTORY

chymosin as a raw material, since **chymosin** retains its enzymatic activity upon extraction from the stored seed.  
Dwg.0/8

L6 ANSWER 2 OF 5 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1998:728582 CAPLUS  
DOCUMENT NUMBER: 130:11268  
TITLE: Manufacture of proteins as **fusion** products  
with zymogen propeptides for processing of  
**fusion** products  
INVENTOR(S): Moloney, Maurice; Alcantara, Joenel; Van Rooijen, Gijs  
PATENT ASSIGNEE(S): Sembiosys Genetics Inc., Can.  
SOURCE: PCT Int. Appl., 44 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9849326	A1	19981105	WO 1998-CA398	19980423
W:	AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
AU 9870240	A1	19981124	AU 1998-70240	19980423
AU 742794	B2	20020110		
EP 977873	A1	20000209	EP 1998-916746	19980423
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI			
BR 9809416	A	20000613	BR 1998-9416	19980423
JP 2001527400	T2	20011225	JP 1998-546439	19980423
ZA 9803471	A	19981027	ZA 1998-3471	19980424
MX 9909801	A	20000331	MX 1999-9801	19991025
PRIORITY APPLN. INFO.:			US 1997-44254P	P 19970425
			WO 1998-CA398	W 19980423

AB An improved method for manuf. of proteins in a foreign host as a **fusion** product is described. The method involves synthesis of the protein as a **fusion** product with a **pro-peptide** of an autocatalytically maturing zymogen that does not process itself in the expression host but that will process in the target organism. The **fusion** protein can then be administered to the host where it will be processed to release the protein of interest. This avoids the need to purify the **fusion** protein, cleave it and sep. the cleavage products. Alternatively, an expression construct for the **fusion** protein can be introduced directly into the target organism. The **pro-peptide**-polypeptide **fusion** protein can be cleaved and the recombinant polypeptide released under the appropriate conditions, e.g as a feed additive that is activated in the stomach. A chimeric gene for a **fusion** protein of hirudin, glutathione-S-transferase and the **pro-peptide** of **chymosin** B was constructed using the pGEX expression system and the protein manufd. in *Escherichia coli*. A significant fraction (5-10%) of the protein accumulated in the cytoplasm with the remainder in inclusion bodies. Incubation of the sol. fraction with **chymosin** at pH 4.5 resulted in the appearance of an anti-thrombin activity. Incubation at pH 2.0 did not lead to processing of the **fusion** protein. A **fusion** protein of **prochymosin** and carp growth hormone was accurately processed by exts. of the gut of the red turnip beetle *Entomoscelis americana*.

REFERENCE COUNT: 2 THERE ARE 2 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L6 ANSWER 3 OF 5 MEDLINE

ACCESSION NUMBER: 1998206747 MEDLINE  
DOCUMENT NUMBER: 98206747 PubMed ID: 9546670  
TITLE: One-step purification of cathepsin D by affinity chromatography using immobilized **propeptide** sequences.  
AUTHOR: Wittlin S; Rosel J; Stover D R  
CORPORATE SOURCE: Novartis Pharma AG, Oncology, Basel, Switzerland..  
sergio.wittlin@pharma.novartis.com  
SOURCE: EUROPEAN JOURNAL OF BIOCHEMISTRY, (1998 Mar 15) 252 (3)

530-6.

Journal code: 0107600. ISSN: 0014-2956.

PUB. COUNTRY: GERMANY: Germany, Federal Republic of  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199805  
 ENTRY DATE: Entered STN: 19980520  
 Last Updated on STN: 19980520  
 Entered Medline: 19980513

AB In vivo, active cathepsin D proteinase is generated by removal of a 44-residue propeptide at its N-terminus. Here we report that mature cathepsin D and pseudocathepsin D (a partially activated form of cathepsin D with 25 amino acid residues removed from the propeptide) bind to the immobilized propeptide, while procathepsin D does not. The N-terminal 25 amino acid residues of the propeptide are sufficient for this binding. Based on this observation, a simple one-step procedure was developed to purify mature cathepsin D from whole cell extracts to near homogeneity. This method has the advantage over existing affinity-purification systems that active forms of the proteinase can be separated from inactive precursors and other aspartic proteinases. Furthermore, this technique was effective for pepsin as well, suggesting it may have general utility for all activated aspartic proteinases and perhaps other families of proteinases.

L6 ANSWER 4 OF 5 MEDLINE DUPLICATE 1

ACCESSION NUMBER: 90358500 MEDLINE  
 DOCUMENT NUMBER: 90358500 PubMed ID: 2117879  
 TITLE: The prepro-peptide of Mucor rennin directs the secretion of human growth hormone by *Saccharomyces cerevisiae*.  
 AUTHOR: Hiramatsu R; Yamashita T; Aikawa J; Horinouchi S; Beppu T  
 CORPORATE SOURCE: Department of Agricultural Chemistry, Faculty of Agriculture, University of Tokyo, Japan.  
 SOURCE: APPLIED AND ENVIRONMENTAL MICROBIOLOGY, (1990 Jul) 56 (7) 2125-32.  
 Journal code: 7605801. ISSN: 0099-2240.  
 PUB. COUNTRY: United States  
 DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)  
 LANGUAGE: English  
 FILE SEGMENT: Priority Journals  
 ENTRY MONTH: 199009  
 ENTRY DATE: Entered STN: 19901026  
 Last Updated on STN: 19901026  
 Entered Medline: 19900927

AB An aspartic proteinase, *Mucor pusillus* rennin (MPR), of filamentous fungus *Mucor pusillus*, is efficiently secreted from a transformant of *Saccharomyces cerevisiae* containing the intact MPR gene. To test the usefulness of the MPR leader peptide in secretion of heterologous proteins from yeast cells, several plasmids encoding the fusion proteins composed of different parts of the NH<sub>2</sub>-terminal region of prepro-MPR and human growth hormone (hGH) were constructed. The parts of the leader peptide upstream of hGH were the whole prepro-peptide following the NH<sub>2</sub>-terminal region of mature MPR in JGH1, the intact pre-sequence and a part of the pro-sequence in JGH2, and the putative signal sequences of the NH<sub>2</sub>-terminal 18 and 22 amino acids in JGH3 and JGH7, respectively. When the hGH genes fused to these leader sequences were expressed in yeast cells under the control of the yeast GAL7 promoter, proteins of various sizes immunoreactive with the anti-hGH antibody were secreted into the medium. Among the plasmids mentioned above, JGH2 directed the greatest secretion of the protein of 23 kilodaltons in size, which contained the expected NH<sub>2</sub>-terminal amino acid sequence of an additional eight amino acids derived from the pro-peptide of MPR. The addition of the GAL10 terminator downstream of the hGH gene in JGH2 resulted in a greater than three- to fivefold increase in the secretion, whereas the insertion of the GAL4 gene, which is a positive regulator for the GAL system, had no significant effect. The improved yield of the total protein of hGH secreted into the medium reached approximately 10 mg/liter.

L6 ANSWER 5 OF 5 CAPLUS COPYRIGHT 2003 ACS

ACCESSION NUMBER: 1987:208790 CAPLUS  
 DOCUMENT NUMBER: 106:208790  
 TITLE: Controlled expression and secretion of bovine chymosin in *Aspergillus nidulans*  
 AUTHOR(S): Cullen, Daniel; Gary, Gregory L.; Wilson, Lori J.; Hayenga, Kirk J.; Lamsa, Michael H.; Rey, Michael W.; Norton, Shirley; Berka, Randy M.

09/402,488 SEARCH RESULTS/HISTORY

CORPORATE SOURCE: Genencor, Inc., South San Francisco, CA, 94080, USA  
SOURCE: Bio/Technology (1987), 5(4), 369-76  
CODEN: BTCHDA; ISSN: 0733-222X  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB To test the ability of the filamentous fungus *A. nidulans* to secrete bovine **prochymosin**, four plasmids were constructed in which the transcriptional, translational, and secretory control regions of the *A. niger* glucoamylase gene were functionally coupled to either **prochymosin** or **preprochymosin** cDNA. Three plasmid constructions involved the in-frame fusion of **prochymosin** coding sequences to glucoamylase sequences at (1) the glucoamylase signal peptide cleavage site, (2) the glucoamylase **propeptide** cleavage site, or (3) after 11 codons of the mature glucoamylase. In a fourth construction, **preprochymosin** was directly fused to the glucoamylase promoter. In all four constructions, the glucoamylase terminator was fused to the 3' end of the **prochymosin** coding sequence. Secretion of polypeptides enzymically and immunol. indistinguishable from bovine **chymosin** [9001-98-3] was achieved following transformation of *A. nidulans* with each of these plasmids. In all cases the primary translation product was partially processed to a polypeptide having a mol. wt. similar to that of bovine **chymosin**. Synthesis of the **chymosin** polypeptides was induced in a medium that contained starch as the sole carbon source, whereas little or no expression was detected when xylose was the sole carbon source. Immunol. assays indicated that the majority (>90%) of **chymosin** was extracellular. Hybridization anal. of genomic DNA from **chymosin** transformants showed chromosomal integration of **prochymosin** sequences, and for some transformants, multiple copies were obsd.